Optimization of Preparation of Oregano Oil Microspheres by Box-Behnken Response Surface Methodology

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Abstract [**Objectives**] To optimize the formulation and preparation of oregano oil microspheres by Box-Behnken response surface methodology. [**Methods**] Chitosan was used as the carrier material to prepare oregano oil microspheres by emulsion crosslinking method. The encapsulation efficiency, drug loading and ID_{50} were used as the evaluation indicators, and the comprehensive score (*OD*) obtained by "coefficient of variation-AHP comprehensive weighting method" was used as the final evaluation indicator. The formulation design and preparation process were optimized by single factor experiment and Box-Behnken response surface methodology, and the optimal process parameters were determined. [**Results**] The optimal formulation and preparation process parameters of oregano oil microspheres were as follows: the ratio of oregano oil to chitosan was 2∶1, the emulsifying speed of double emulsion was 200 r/min, the amount of emulsifier in the colostrum was 4%, and the volume of curing agent was 1.0 mL. The average encapsulation efficiency was 45.33% ±1.32%, the average drug loading was 30.59% ±2.45%, and the median diameter (ID_{50}) was 52.596 μm ±0.023%. [**Conclusions**] The encapsulation efficiency, drug loading and ID_{50} of oregano oil chitosan microspheres prepared by emulsion crosslinking method met the requirements. The drug-loaded microsphere not only can be used as a preparation finished product for direct application, but also be used as a product intermediate to lay a foundation for the research and development of subsequent dosage forms.

Key words Oregano oil, Chitosan, Microspheres, Preparation, Response surface methodology (RSM)

1 Introduction

Oregano oil is a kind of volatile oil extracted from Chinese herbal medicine *Origanum vulgare* L.[1-4]. It is yellow or brownish yellow oily liquid at room temperature and has specific aroma. Its main components are carvacrol, thymol, p-cymene, caryophyllene, and so on[5]. Studies have shown that oregano oil has good antibacterial, anti-inflammatory, antioxidant and other biological activities[6-7]. Previous studies of our team found that oregano oil can improve the symptoms of ulcerative colitis (UC) by inhibiting inflammatory factors, regulating intestinal flora and repairing intestinal mucosal barrier[8]. However, the poor stability, strong irritation and weak formation of oregano oil limit its dosage form development and clinical application.

Microspheres are tiny spherical entities formed by dispersing or adsorbing drugs in a polymer matrix, and their particle size is generally 1 - 500 μm[9]. Microspheres can not only be used as intermediates to lay the foundation for the development of dosage forms, but also be dispersed in water or hydrophilic matrix for direct application as injection or oral preparations[10]. In addition, microspheres can reduce the irritation of essential oil of traditional Chinese medicine and enhance its stability[11-12]. Therefore, in this study, we used chitosan with biodegradability as the dispersion material to prepare oregano oil loaded microspheres, to some experimental reference and basis for the prevention and treatment of UC and the subsequent dosage form development of oregano oil.

2 Materials and methods

2.1 Materials

2.1.1 Instruments. SQP analytical balance (Sartorius Scientific Instruments (Beijing) Co., Ltd.); HH-2 water bath (Changzhou Guohua Electric Appliance Co., Ltd.); HH-6J magnetic stirring water bath (Guizhou Enpei Instrument Manufacturing Co., Ltd.); BT-1000 powder integrated characteristic tester (Dandong Better Size Instrument Co., Ltd.); MS-2000 laser particle sizer (Malvern Panalytical); Agilent 1260 liquid chromatograph (Agilent Technologies, Inc.).

2.1.2 Reagents. Chitosan (Sinopharm Chemical Reagent Co., Ltd., 20231027); Glutaraldehyde (McLean Chemicals, C13576856); corn oil (Shandong Sanxing Corn Industry Technology Co., Ltd., SX0401338); anhydrous ethanol (Sinopharm Chemical Reagent Co., Ltd., 2312139); dichloromethane (Sinopharm Chemical Reagent Co., Ltd., 2309182); acetic acid (Sinopharm Chemical Reagent Co., Ltd., 231120); phosphoric acid (Xilong Scientific Co., Ltd., 2312131); oregano oil (self-extracted, oregano collected in June 2023).

2.2 Methods

2.2.1 Method for preparing oregano oil microspheres. Weighed about 0.30 g oregano oil, added a certain amount of soybean oil to dilute to 2.0 mL, add 0.8 g Tween-80 to 20 mL (3%, *W/V*) chi-

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tosan solution, stirred well and added oregano oil-soybean oil solution to chitosan solution. The emulsion was sheared and emulsified for 10 min at the speed of 10 000 r/min in ice water bath. Later, added the prepared colostrum to 100 mL corn oil, stirred at 200 r/min for 2 min, then added 1.0 mL (25%, *W/V*) glutaraldehyde aqueous solution, stirred for 5 – 10 min, and solidified the system in a refrigerator at 4 ℃. After curing, the microspheres were washed with dichloromethane, absolute ethanol and ultrapure water for 5 times separately, and the microspheres were dried in an oven at 40 ℃. Took 0.20 g of the dried microspheres, ground and dissolved in ethanol, and fixed the volume in a 25 mL volumetric flask, injected 10 μL of the sample after ultrasonic treatment, and determined the encapsulation efficiency, drug loading, ID_{50} and comprehensive score.

2.2.2 Chromatographic conditions. Column: Kromasil C_{18} Column (250 mm × 4.6 mm, 5.0 μm); mobile phase: acetonitrile-0.2% phosphoric acid water = 51 ∶ 49, flow rate: 1.0 mL/min; Detection wavelength: 274 nm; Column temperature: (25 ±0.5) ℃; Injection volume: 10 μL;

2.2.3 Preparation of solutions. (i) Mixed standard solution. Weighed 3.00 mg of carvacrol standard and 7.00 mg of thymol standard into a 10 mL volumetric flask, added absolute ethyl alcohol to constant volume, and shooke up to obtain a mixed standard solution containing 0.30 mg/mL of carvacrol and 0.70 mg/mL of thymol.

(ii) Oregano oil-ethanol solution. Weighed 104 mg of oregano oil and put it into a 100 mL volumetric flask, added absolute ethyl alcohol to constant volume to obtain 1.04 mg/mL of oregano oil-ethanol solution.

(iii) Test solution. Weighed 0.50 g of oregano oil microspheres, added a mortar to grind for 10 min, added a small amount of absolute ethanol to continue grinding for 5 min, transferred the supernatant to a 25 mL volumetric flask, added a small amount of ethanol to the mortar again to wash, transferred the supernatant to a volumetric flask, repeated for 3 times, and then fixed the volume with absolute ethanol.

(iv) Negative sample solution. Prepared a batch of blank microspheres without oregano oil according to the method in Section **2.2.1**, weighed an appropriate amount, ground it and put it into a 25 mL volumetric flask.

2.2.4 Specificity test. Separately injected the prepared mixed standard, test sample and negative sample solution according to the above chromatographic conditions, and checked whether the color spectrum peaks are completely separated.

2.2.5 Investigation of linear relationship. Separately injected 0.5, 1.0, 5.0, 10.0, 15.0 and 20.0 μL of the prepared mixed standard solution. The peak areas of carvacrol at 0.015, 0.03, 0.15, 0.3, 0.45 and 0.6 mg/mL and thymol at 0.035, 0.070, 0.350, 0.700, 1.050 and 1.400 mg/mL were obtained, repeated for 3 times and calculated the average value. Plotted the standard curve of carvacrol and thymol with the peak area as the ordinate (Y) and the mass concentration as the abscissa (X).

2.2.6 Determination of carvacrol and thymol in oregano oil. Injected the prepared oregano oil solution for 3 times, 10 μL each time, and substituted the peak areas of carvacrol and thymol into the standard curves of carvacrol and thymol separately to determine the contents of carvacrol and thymol in oregano oil. The proportion of the sum of the two contents in the oregano oil was used as an indicator for detecting the content of the oregano oil in the microspheres.

2.2.7 Single factor experiment of oregano oil microspheres. In accordance with the preparation method of drug-loaded microspheres in Section **2.2.1**, the effects of multiple emulsion emulsification speed, multiple emulsion emulsification time, curing time, primary emulsion emulsification speed, primary emulsion oil-water phase ratio, oregano oil/chitosan dosage ratio, emulsifier dosage and curing agent dosage on the indicator parameters of oregano oil drug-loaded microspheres were investigated.

2.2.8 Response surface experiment of oregano oil microspheres. On the basis of single factor experiment, four factors that have great influence on each indicator parameter of microspheres were selected for response surface experiment. The four factors are: A: multiple emulsion emulsification speed (r/min), B: oregano oil/chitosan dosage ratio, C: colostrum emulsifier dosage (%), D: curing agent dosage (mL). The specific experimental arrangement is shown in Table 1.

Table 1 Box-Behnken response surface factor level

Level	Factor			
	Multiple emulsion emulsification speed r/min (A)	Oregano oil/chitosan dosage ratio (B)	Colostrum emulsifier dosage (C)//%	Curing agent dosage (D)//mL
−1	100	1	1	1
0	200	2	4	2
1	300	3	7	3

The factor level data in Table 2 were imported into DX (Design-Expert 13.0) to design the response surface experiment table.

2.2.9 Evaluation indicators of oregano oil microspheres. (i) Encapsulation efficiency and drug loading. Pipetted 1.0 mL of the test solution, filtered it with a 0.22 μm microporous membrane, and transferred it to a liquid phase vial. Detected the peak area at the injection volume of 10 μL, and then calculated the encapsulation efficiency (EE) according to Equation (1), and the drug loading (DL) according to Equation (2).

$$EE\ (\%) = W_1/W_2 \times 100\% \quad (1)$$

where W_1 denotes the content of oregano oil in the microspheres, and W_2 denotes the addition amount of oregano oil.

$$DL(\%) = W_1/W_3 \times 100\% \quad (2)$$

where W_1 denotes the content of oregano oil in the microspheres, and W_3 denotes the total weight of the drug-loaded microspheres.

(ii) Determination of median particle size (ID_{50}). Weighed an appropriate amount of drug-loaded microspheres and added into Malvern laser particle size analyzer, and measured the median

particle size (ID_{50}) and particle size span (PDI) by dry method.

(iii) Determination of comprehensive score by the variation coefficient method and the AHP compound weighting method.

(a) Weight determination by variation coefficient method.

$$V_i = \delta_i / \bar{x}_i \quad (3)$$

where V_i is the variation coefficient of the i^{th} indicator; δ_i is the standard deviation of the i^{th} indicator; $\bar{x}_i$ is the average of the i^{th} indicator.

$$W_i = V_i / (V_1 + V_2 + V_3) \quad (4)$$

where W_i is the weight of the i^{th} indicator.

(b) Determination of weights by AHP. There are three indicator parameters of microspheres, namely, encapsulation efficiency (EE), drug loading (DL) and ID_{50}, which are recorded as indicator 1, 2, and 3, respectively. First, the scoring criteria of each indicator level were determined (Table 2).

Table 2 Scoring criteria for each level

Relative importance	Definition of importance
5	Highly important
3	Generally important
1	Equally important

The encapsulation efficiency (EE) and drug loading (DL) of microspheres were more important than ID_{50}. Set the ratio of indicator 1: indicator 2: indicator 3 as 3: 3: 1, and input this ratio into SPSS AU software to obtain the AHP weight of each indicator, as indicated in Table 3.

Table 3 Comparison priority judgment matrix of three evaluation indicators in pairs

Target	EE	DL	ID_{50}	AHP weight	Consistency test
EE	1	1	3	0.428 57	Pass
DL	1	1	3	0.428 57	
ID_{50}	1/3	1/3	1	0.142 86	

(c) Compound weight. Based on the coefficient of variation of each indicator and the AHP weight, the composite weight was determined according to Equation (5).

$$W_{\text{compound } i} = (W_{\text{variation i}} \times W_{Ai}) / [(W_{\text{variation 1}} \times W_{A1}) + (W_{\text{variation 2}} \times W_{A2}) + (W_{\text{variation 3}} \times W_{A3})] \quad (5)$$

where $W_{\text{compound } i}$ denotes the compound weight of the i^{th} indicator, $W_{\text{variation } i}$ denotes the weight of the coefficient of variation of the i^{th} indicator, and W_{Ai} is the weight of the AHP coefficient of the i^{th} indicator.

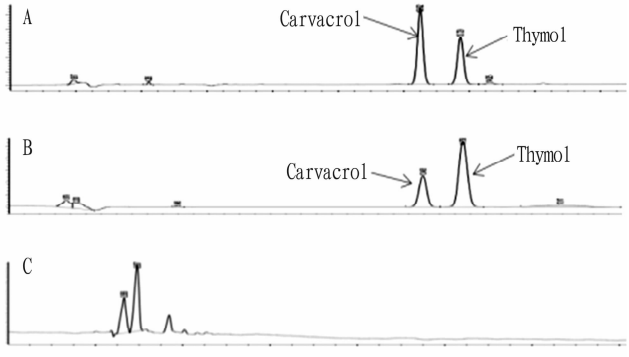
(d) Comprehensive score (OD value). Based on the compound weight score, the comprehensive score of each group was determined according to Equation (6).

$$OD = [(W_{\text{compound 1}} \times Y_1) / Y_{1\max}] + [(W_{\text{compound 2}} \times Y_2) / Y_{2\max}] - [(W_{\text{compound 3}} \times Y_3) / Y_{3\max}] \quad (6)$$

In the equations (3) to (6), the OD value is the comprehensive score of each group of microspheres, $W_{\text{compound 1}}$, $W_{\text{compound 2}}$ and $W_{\text{compound 3}}$ are the compound weights of the first, second and third indicators, Y_1, Y_2 and Y_3 are the numerical values of the first, second and third indicators, and $Y_{1\max}$, $Y_{2\max}$ and $Y_{3\max}$ are the maximum values of the first, second and third indicators.

3 Results and analysis

3.1 Specificity test results As shown in Fig. 1, the chromatographic peaks of oregano oil, mixed standard and negative sample solution were well separated, and other peaks had no effect on the peak areas of carvacrol and thymol, indicating that the specificity of carvacrol and thymol met the requirements.



NOTE A. Oregano oil sample solution; B. Mixed standard solution; C. Negative control solution.

Fig. 1 Liquid chromatogram of oregano oil, mixed standard and negative control solution

3.2 Standard curve The regression equation of carvacrol standard curve was $Y = 7\ 558X + 3.904\ 5$ ($R^2 = 1$), indicating that carvacrol had a good linear relationship in the range of 0.03 – 0.60 mg/mL; the regression equation of thymol standard curve was $Y = 7\ 518X - 0.665\ 8$ ($R^2 = 1$), indicating that thymol had a good linear relationship in the range of 0.07 – 1.40 mg/mL.

3.3 Determination of carvacrol and thymol in oregano oil First, injected the 1.04 mg/mL oregano oil ethanol solution for 3 times continuously, with the injection volume of 10 μL each time. Substituted the peak areas of carvacrol and thymol into their standard curves, and calculated the contents of carvacrol and thymol in oregano oil. The results are shown in Table 4.

Table 4 Percentage of carvacrol combined with thymol in oregano oil

Number of injections	Component	Retention time // min	Peak area	Injection volume // μL	Sum of carvacrol and thymol concentration // mg/mL	Oregano oil concentration // mg/mL	Mean ± RSD
1	Carvacrol	12.875	1 826.94	10	0.389 5	1.04	37.46% ±0.14%
	Thymol	14.160	1 231.57	10			
2	Carvacrol	12.856	1 829.97	10	0.390 2		
	Thymol	14.115	1 233.54	10			
3	Carvacrol	12.880	1 824.85	10	0.389 1		
	Thymol	14.174	1 230.02	10			

It can be seen from the data in the table that the proportion of carvacrol and thymol in oregano oil was 37.46%, and the *RSD* was 0.14% <3%, which meets the relevant requirements for content determination.

3.4 Single factor experiment results of oregano oil microspheres

3.4.1 Effects of multiple emulsion emulsification speed on microspheres. It can be seen from the data in Table 5 that when the multiple emulsion emulsification speed is 200 r/min, the indicator parameters were significantly better than those of other groups, and with the increase of the rotating speed, the indicator parameters were significantly reduced. Thus, the multiple emulsion emulsification of 200 r/min was the optimum process.

Table 5 Effects of multiple emulsion emulsification speed on indicators of microspheres

Multiple emulsion emulsification speed//r/min	*EE*//%	*DL*//%	ID_{50}//μm	*OD* value
200	13.1	3.89	105.768	0.812 4
300	1.47	0.45	215.24	0.061 5
400	1.47	5.51	330.611	0.424 3
500	0.27	0.1	241.79	-0.018 2
600	0	0	120.845	-0.018 2
700	0	0	81.793	-0.012 3

3.4.2 Effects of emulsification time on microspheres. It can be seen from the data in Table 6 that when the emulsification time was 2 min, the indicator parameters were significantly better than those of other groups, so the optimal process was tentatively determined as the emulsification time of 2 min.

Table 6 Effects of multiple emulsion emulsification speed on indicators of microspheres

Emulsification time//min	*EE*//%	*DL*//%	ID_{50}//μm	*OD* value
0	25.06	5.6	353.962	0.521 1
1	25.2	5.85	77.786	0.504 1
2	26.77	8.87	46.204	0.752 8
4	3.4	0.91	174.852	0.016 5
6	1.43	0.45	175.099	-0.025 4
8	23.29	5.55	198.177	0.429 3

3.4.3 Effects of curing time on microspheres. It can be seen from the data in Table 7 that when the solidification time was less than 4 h, the solidification was incomplete, the encapsulation efficiency and drug loading were low, and part of the unsolidified colostrum was filtered; when the curing time was longer than 6 h, the microspheres had a certain degree of adhesion, and with the increase of curing time, the degree of adhesion became more serious, the encapsulation efficiency and drug loading decreased significantly, and the particle size of microspheres increased. Therefore, the best curing time is 4 - 6 h, and the encapsulation efficiency, drug loading, *OD* value and ID_{50} value of the microspheres were higher and smaller after curing for 6 h, so the optimal process condition was temporarily determined to be 6 h.

Table 7 Effects of curing time on indicators of microspheres

Curing time//h	*EE*//%	*DL*//%	ID_{50}//μm	*OD* value
2	6.47	2.47	74.494	0.170 2
4	25.15	10.06	73.423	0.781 3
6	27.15	11.39	61.901	0.873 1
8	3.76	1.41	208.433	0.020 3
10	3.84	1.84	80.211	0.099 2
12	12.14	4.22	123.919	0.310 1

3.4.4 Effects of oregano oil/chitosan dosage ratio on microspheres. It can be seen from the data in Table 8 that when the oregano oil/chitosan dosage ratio was 2∶1, all indicator parameters were significantly better than those of other groups, so the oregano oil/chitosan dosage ratio of 2∶1 was tentatively determined as the optimal process condition.

Table 8 Effects of oregano oil/chitosan dosage ratio on indicators of microspheres

Oregano oil/chitosan	*EE*//%	*DL*//%	ID_{50}//μm	*OD* value
0.25	8.5	1.76	57.007	0.090 9
0.50	25.41	11.94	619.634	0.151 6
1.00	18.14	20.31	35.187	0.403 4
1.5	12.5	17.36	68.484	0.294 5
2.00	21.62	43	88.101	0.657 8
3.00	3.84	7.36	55.6	0.097 0

3.4.5 Effects of the amount of emulsifier in the colostrum on the microspheres. It can be seen from the data in Table 9 that when the dosage of Tween 80 was 4% (*W*/*V*), that is, 4% of the chitosan solution, the indicator parameters were significantly better than those of other groups, so amount of emulsifier of 4% was temporarily determined as the optimal process condition.

Table 9 Effects of the amount of emulsifier in the colostrum on indicators of microspheres

Tween 80 dosage//%	*EE*//%	*DL*//%	ID_{50}//μm	*OD* value
0.5	2.38	4.94	83.175	0.064 7
1	0.33	0.62	57.899	-0.009 1
2	0.85	1.28	76.351	0.000 3
3	15.27	24.75	67.442	0.516 7
4	26.8	45.03	65.557	0.943 9
5	25.37	44.83	45.484	0.923 5

3.4.6 Effect of the amount of curing agent on microspheres. It can be seen from the data in Table 10 that when the dosage of 25% (*W*/*V*) of glutaraldehyde was less than 1.00 mL, the curing was incomplete and the microspheres cannot be formed. When the dosage was greater than 1.00 mL, the microspheres can be cured, and when the dosage of glutaraldehyde was 1.00 mL, the indicator parameters were significantly better than those of other groups, so the optimal curing agent dosage was determined as 1.00 mL.

Table 10 Effects of the amount of curing agent on indicators of microspheres

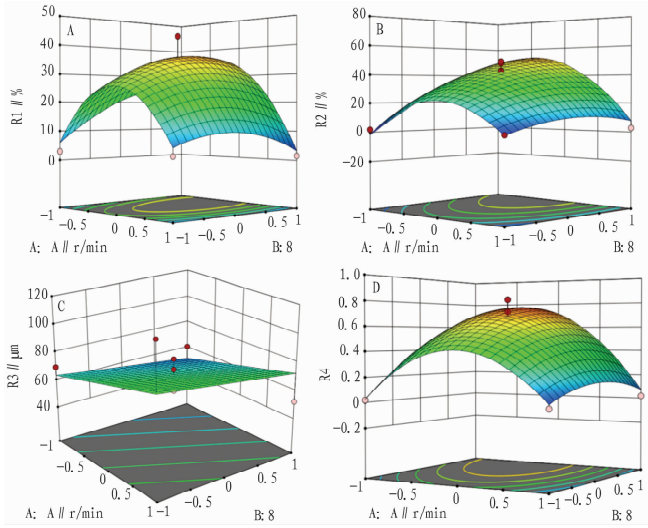
Amount of curing agent//mL	*EE*//%	*DL*//%	ID_{50}	*OD* value
0.25	0	0	–	–
0.50	0	0	–	–
1.00	31.98	46.25	45.889	0.930 2
1.50	24.83	36.68	84.821	0.704 0
2.00	23.48	28.75	67.177	0.610 5
3.00	16.36	21.10	73.659	0.422 1

3.5 Box-Behnken response surface analysis of oregano oil microspheres The response surface experiment was carried out according to the arrangement of oregano oil microsphere response surface experiment, and the encapsulation efficiency (*EE*, %), drug loading (*DL*, %), ID_{50} and *OD* value of each group of microspheres were measured. The results are shown in Table 11.

Table 11 Box-Behnken response surface experiment results

No.	A	B	C	D	*EE*//%	*DL*//%	ID_{50}//μm	*OD* value
1	0	0	−1	−1	0.78	1.57	79.108	−0.001 0
2	1	0	−1	0	0.58	0.93	63.990	−0.011 5
3	0	−1	1	0	19.84	13.30	67.974	0.289 2
4	0	0	0	0	42.98	48.78	63.104	0.817 6
5	0	1	0	1	31.01	41.23	75.749	0.625 9
6	0	−1	−1	0	0.74	0.69	82.197	−0.018 8
7	−1	0	0	1	25.62	26.37	62.810	0.456 1
8	0	0	0	0	34.56	47.59	71.712	0.715 5
9	0	1	1	0	28.24	34.61	66.613	0.547 7
10	0	0	1	−1	35.87	56.83	54.217	0.809 5
11	0	1	0	−1	32.70	62.78	42.209	0.827 7
12	0	−1	0	1	17.26	9.94	53.999	0.240 7
13	0	1	−1	0	10.95	24.02	66.114	0.280 6
14	0	0	0	0	30.79	29.87	78.802	0.532 3
15	1	0	0	1	4.16	4.43	118.570	0.032 9
16	0	0	−1	1	0.30	0.55	63.899	−0.017 4
17	−1	1	0	0	23.56	43.87	60.643	0.573 8
18	−1	0	−1	0	4.46	7.91	63.947	0.085 0
19	1	1	0	0	1.56	2.92	51.928	0.019 3
20	−1	0	0	−1	19.89	30.72	51.862	0.434 1
21	0	0	0	0	33.79	42.47	58.039	0.672 1
22	1	−1	0	0	3.45	2.33	119.727	0.008 2
23	0	−1	0	−1	32.39	20.37	45.546	0.487 1
24	1	0	1	0	2.17	2.76	70.009	0.017 5
25	1	0	0	−1	1.32	2.09	87.890	−0.003 9
26	−1	0	1	0	3.63	6.24	72.769	0.059 4
27	−1	−1	0	0	2.91	1.89	69.609	0.018 6
28	0	0	1	1	21.48	24.89	60.490	0.401 3
29	0	0	0	0	32.56	42.82	55.741	0.662 8

3.5.1 Response surface analysis of oregano oil microspheres. The relationship between the four factors A, B, C and D and the indicator parameters R1 (encapsulation efficiency), R2 (drug loading), R3 (ID_{50}) and R4 (*OD* value) was observed using Design-Expert 13.0. The results were as follows. It can be seen from the 3D effect diagram of each indicator that the four factors had significant effects on the encapsulation efficiency, drug loading and *OD* value of oregano oil-chitosan microspheres, and there were extreme values in the experimental range, indicating that the interaction of the four factors was significant (Fig. 2). The results were consistent with the analysis of variance of each indicator parameter. The four factors had no significant effect on the ID_{50} of microspheres, but the ID_{50} of most microspheres was in the range of 30 – 60 μm, which was in line with the required range of particle size of microspheres.



NOTE A, B, C, and D are the effects of each factor on encapsulation efficiency, drug loading, ID_{50} and *OD* value of oregano oil microspheres.

Fig. 2 Response surface analysis of each factor on oregano oil microspheres

3.5.2 Results of variance analysis. It can be seen from the variance analysis results of *OD* values in Table 12 that the experimental model was significant ($P < 0.01$), and the lack of fit was not significant ($P = 0.151\ 3$), indicating that the model was true and credible. Factors A, B and C had significant effects on the microsphere comprehensive score (*OD* value) ($P < 0.05$), while factor D had no significant effect on the *OD* value ($P > 0.05$). According to the results of the optimal process for microsphere preparation ($n = 100$), the optimal process conditions were selected as follows: multiple emulsion emulsification speed 200 r/min, oregano oil: chitosan = 2: 1, emulsifier 4%, curing agent 2.0 mL. Three batches of microspheres were prepared according to the optimal process, and the encapsulation efficiency (%), drug loading (%) and ID_{50} were measured, as shown in Table 13. Under these conditions, the encapsulation efficiency of prepared microspheres was 45.33% ±1.32%, the drug loading was 30.59% ±2.45%, the ID_{50} was 52.596 μm, and the *RSD* was less than 3%. The results showed that the optimal process parameters were suitable for the preparation of oregano oil microspheres with good reproducibility.

Table 12 Variance analysis of *OD* value

Source	Sum of squares	*df*	Mean square	*F*	*P*	Significance
Model	2.23	14	0.15	6.21	0.000 8	Extremely significant
A	0.20	1	0.20	7.95	0.013 6	Significant
B	0.29	1	0.28	11.12	0.004 9	Significant
C	0.27	1	0.27	10.61	0.005 7	Significant
D	0.05	1	0.05	2.15	0.164 3	Not significant
Residual error	0.35	14	0.02			
Lack of fit	0.31	10	0.03	2.99	0.151 3	Not significant
Pure error	0.04	4	0.01			
Total sum		28				

Table 13 Optimal process results

Group	*EE*//%	*DL*//%	ID_{50}//μm
Optimal process 1	46.01	30.15	53.999
Optimal process 2	44.89	31.46	51.928
Optimal process 3	45.08	30.16	51.862
Mean	45.33	30.59	52.596
RSD//%	1.32	2.45	2.310

4 Conclusions and discussion

In this study, we used the microspheres to encapsulate oregano oil in the carrier material, which enhanced its stability and reduced the irritation of traditional Chinese medicine essential oil, and laid a certain foundation for the release of oregano oil preparation in the colon. During the experiment, Box-Behnken response surface methodology was used to optimize the formulation design and preparation process of oregano oil microspheres, and the comprehensive score of each factor level was determined by combining coefficient of variation-AHP composite weighting method. The method not only has subjective selection and judgment, but also respects the objective rules, and the obtained optimal process parameters have certain application value. This study has not been verified by pharmacodynamics, and the anti-inflammatory effect of oregano oil microspheres can not be determined, which can be supplemented after the completion of the future preparation development. However, oregano oil microspheres can be used as product intermediates and preparations directly. This study provides some experimental reference and reference for the development of dosage forms of essential oil of traditional Chinese medicine. The encapsulation efficiency and drug loading of oregano oil microspheres obtained in this experiment were low, which may be due to the unstable physical and chemical properties of oregano oil, but they could be moderately improved by changing the experimental conditions and methods. Therefore, the encapsulation efficiency and drug loading of oregano oil microspheres need to be further studied.

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